Bulletin of Entomological Research (2006) **96**, 137–144 DOI: 10.1079/BER2005409

Genetic differentiation among various populations of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae)

A. Pichon[1], L. Arvanitakis[2], O. Roux[1], A.A. Kirk[3], C. Alauzet[1], D. Bordat[2] and L. Legal[1]*

[1]Laboratoire dynamique de la biodiversité, UMR UPS/CNRS 5172, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse Cedex 4, France: [2]CIRAD/AMIS, Laboratoire Entotrop, CSIRO Campus International de Baillarguet, TA 40/L, 34398 Montpellier Cedex 5, France: [3]USDA/ARS, European Biological Control Laboratory, Montferrier sur Lez, 34398 St Gély du Fesc, France

Abstract

Genetic variation among 14 populations of *Plutella xylostella* (Linnaeus) from USA (Geneva, New York), Brazil (Brasilia), Japan (Okayama), The Philippines (Caragan de Oyo), Uzbekistan (Tashkent), France (Montpellier), Benin (Cotonou), South Africa (Johannesburg), Réunion Island (Montvert), and five localities in Australia (Adelaide, Brisbane, Mareeba, Melbourne, Sydney) were assessed by analysis of allozyme frequencies at seven polymorphic loci. Most of the populations were not in Hardy–Weinberg equilibrium and had a deficit in heterozygotes. The global differentiation among populations was estimated by the fixation index (Fst) at 0.103 for the 14 populations and at 0.047 when populations from Australia and Japan, which differed most and had a strong genetic structure, were excluded from the analysis. By contrast, the populations from Benin (West Africa) and Brazil (South America) were very similar to each other. Genetic differentiation among the populations was not correlated with geographical distance.

Keywords: *Plutella xylostella*, allozyme, population differentiation

Introduction

Plutella xylostella (Linnaeus) (Lepidoptera: Yponomeutidae), the diamondback moth, is a major pest of *Brassica* crops and has a worldwide distribution (Talekar & Shelton, 1993). Its geographical origin is considered to be in the eastern Mediterranean region or South Africa because of the number of wild and endemic brassicas in these regions and the presence of a high number of known parasitoid species (Tsunoda, 1980; Kfir, 1998).

*Author for correspondence
Fax: 00 33 (0)5 61 55 61 96
E-mail: legal@cict.fr

Populations of diamondback moth in tropical areas cause severe damage (as in Southeast Asia), but in temperate areas (e.g. Canada, USA, UK) damage to crops is usually less dramatic (Lim, 1986). Measures to control this pest were estimated globally at US$ 1 billion in 1992 (Talekar & Shelton, 1993). The main strategy of management has traditionally been insecticides (Talekar & Shelton, 1993). As a major consequence, resistance to synthetic insecticides has appeared in a relatively short period of time in the field (Sun *et al*., 1986). In addition *P. xylostella* has become the first species to develop field resistance to some of the toxins of *Bacillus thuringiensis* Berliner (Eubacteriales) (Tabashnik *et al*., 1987; Talekar & Shelton, 1993).

Genetic differences and gene flow between populations of *P. xylostella* have been investigated in the context of

Table 1. Location and date of collection of *Plutella xylostella*.

Country	Locality	Latitude	Longitude	Abbreviation	Date of collection
South Africa	Johannesburg	26°08′ S	27°54′ E	SA	07/1999
Benin	Cotonou	6°29′ N	2°37′ E	BEN	09/1998
Réunion Island	Montvert	20°52′ S	55°28′ E	REU	08/1998
Brazil	Brasilia	15°52′ S	47°55′ W	BRA	08/1998
United States	Geneva, NY	42°51′ N	76°59′ W	USA	08/1999
France	Montpellier	43°38′ N	3°53′ E	FRA	08/1998
Uzbekistan	Tashkent	41°19′ N	69°15′ E	UZB	06/1999
Japan	Okayama	34°39′ N	133°55′ E	JAP	07/1999
The Philippines	Caragan de Oyo	7°04′ N	125°36′ E	PHI	01/1999
Australia	Adelaide	34°46′ S	138°32′ E	AUAd	06/1999
Australia	Brisbane	27°30′ S	153°10′ E	AUBr	05/1999
Australia	Mareeba	17°00′ S	145°26′ E	AUMa	08/1999
Australia	Melbourne	37°52′ S	145°08′ E	AUMe	03/1999
Australia	Sydney	33°55′ S	151°17′ E	AUSy	06/1999

insecticide resistance. Differences have been shown in the level of susceptibility to insecticide between populations less than 10 km apart, both in Taiwan and in Hawaii (Cheng, 1981; Liu *et al*., 1982; Tabashnik *et al*., 1987). These differences were probably induced by local selection pressure due to local variations in the insecticide treatments and the compounds used. Gene flow might not have been sufficient to overcome the differences in insecticide susceptibility between the populations in Hawaii (Tabashnik *et al*., 1987). Therefore it could be assumed that in some regions, isolated populations of *P. xylostella* may differentiate from each other, because of a strong selection pressure and because of reduced migration and therefore reduced gene flow.

However, adults can migrate over very long distances; mass migrations have been reported in temperate climates, between the south of Finland and England (Mackenzie, 1958), The Netherlands and England (Chapman *et al*., 2002) and between the southern USA and Canada (Harcourt, 1986). In these areas, the gene flow may be sufficient to overcome differences between the existing populations; however, differences in levels of insecticide resistance between populations may also result from different selection pressures due to local variation in insecticide use.

The genetic variation among world populations of *P. xylostella* using neutral alleles was not estimated. It was assumed that environmental factors, such as variations of temperature and rainfall could affect the selection pressure. Therefore it could be considered that genetic differences between populations of *P. xylostella* could also occur on genes which were not linked to insecticide resistance. In this study, these genetic differences between widely separated populations of *P. xylostella* were investigated with the objective of determining whether they were related to the geographical distance between populations. An earlier study of the estimate of gene flow from neutral or quasi-neutral allelic variation among populations from Hawaii, Wisconsin and Florida in the USA showed low levels of differentiation and suggested a substantial gene flow occurring between them (Caprio & Tabashnik, 1992). The enzyme electrophoresis technique, used in the study has been used to analyse geographical variations among populations of lepidopterous species (Daly & Gregg, 1985; Buès *et al*., 1994; Wainhouse & Juke, 1997).

The aims of the present study were to measure differences among *P. xylostella* populations worldwide and investigate possible reasons for these differences. Allelic variation was measured in populations from 14 different areas: USA, Brazil, South Africa, Benin, Réunion Island, France, Uzbekistan, Japan, the Philippines and five areas in Australia (Sydney, Brisbane, Melbourne, Mareeba, and Adelaide).

Materials and methods

Sampling

Strains of *P. xylostella* were obtained from 14 localities worldwide (table 1). Each sample comprised a minimum of 100 larvae and pupae. One of us (A. Kirk) and foreign collaborators collected pupae and larvae (second and third instars) by hand from plants from one field at each locality. They were immediately placed in plastic dishes with leaves of their host plant (cabbage) and dispatched by air to the CIRAD laboratory (Centre de Coopération Internationale en Recherche Agronomique pour le Développement, France) within 48 h. Samples received were reared at 25°C, ±2°C, 75% relative humidity, and a photoperiod of 12L/12D on a sequence of cultivated *Brassica* species. Different *Brassica* species were used in order to maintain moths in good condition at each stage of their development. We observed that second instar larvae developed better when placed on fresh leaves of cabbage (*B. oleracea* L. var. Chateaurenard), whereas third instar larvae did best on cauliflower leaves (*B. oleracea* L. var *botrytis*) until pupation. All pupae were placed in a plastic box until emergence of adults (males and females) which were placed in a Plexiglas cage with Chinese mustard *B. juncea* (L.) plant. Chinese mustard was used for oviposition and first instar larval development. When the number of eggs was sufficient to establish a laboratory colony, the adults were deep frozen in liquid nitrogen and conserved at −80°C. If the number of individuals was insufficient for a complete analysis, offspring of the moths were reared under the same conditions for another generation. Adults were deep frozen in liquid nitrogen and conserved at −80°C.

Enzyme electrophoresis

Horizontal starch gels (13%) were prepared following the protocol of Pasteur *et al*. (1987) and stored at 4°C for up to

Table 2. Enzymes screened in populations of *Plutella xylostella* and running conditions for electrophoresis.

Buffer	Enzyme stained	EC	Abbreviation
Tris-citrate pH 8	Isocitrate dehydrogenase	1.1.1.42	IDH
	$NADPH^+$ malate dehydrogenase	1.1.1.40	MDHP
	Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PDH
Histidine pH 6	Mannose-6-phosphate isomerase	5.3.1.8	MPI
	Phosphoglucomutase	5.4.2.2	PGM
Tris-maleate EDTA pH 7.4	Hexokinase	2.7.1.1	HK
	Adenylate kinase	2.7.4.3	AK
	Acid phosphatase	3.1.3.2	ACP
	Glucose-6-phosphate isomerase	5.3.1.9	GPI
	Glutamate dehydrogenase	1.4.1.2	GTDH
	Hydroxybutyrate dehydrogenase	1.1.1.30	HBDH
	Phosphogluconate dehydrogenase	1.1.1.44	PGDH
	Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH
	(S)-2-hydroxy-acid-oxidase	1.1.3.15	HAOX
Tris-ctrate pH 8.7	Aspartate amino transferase	2.6.1.1	AAT
	Superoxide dismutase	1.15.1.1	SOD
	Glucose dehydrogenase	1.1.1.118	GCDH
	Xanthine dehydrogenase	1.1.1.204	XDH
	Alcohol dehydrogenase	1.1.1.1	ADH
	Gluthatione reductase	1.6.4.2	GR
	Pyruvate kinase	2.7.1.40	PK

one day. Gel buffers used were Tris-citrate pH 8, histidine pH 6, Tris-citrate pH 8.7, Tris-maleate-EDTA pH 7.4.

Each entire frozen adult was homogenized in 20 µl of a NADP 2.3% solution using a pestle and mortar. This solution was then centrifuged for 10 min at 13000 rpm and 4°C. Fifteen µl of the resulting supernatant was maintained at −20°C or used immediately.

Each sample (15 µl of supernatant) was loaded on a wick of Whatman no. 3 filter paper and placed into a well of the gel. Each gel consisted of 15 wells equivalent to 15 adults from five populations, therefore three adults per population. Migration (150 V, 70 mA) lasted 6 h at 4°C. Migration buffers used were: Tris-citrate pH 8, Tris-maleate EDTA pH 7.4, borate/NaOH pH 8.25, Tris-citrate pH 8.7 (Pasteur *et al*., 1987; Hillis *et al*., 1996).

Soon after migration, each gel was horizontally sliced to four layers, 2 mm thick. Stains were prepared as required following the procedure of Pasteur *et al*. (1987) and Hillis *et al*. (1996). Different enzymes were tested (table 2) and each enzyme was stained in one slice. The following seven enzymes exhibited discernable bands and polymorphic loci: isocitrate dehydrogenase, $NADPH^+$ malate dehydrogenase, glucose-6-phosphate dehydrogenase, mannose-6-phosphate isomerase, phosphoglucomutase, hexokinase and aspartate aminotransferase. The banding patterns characteristic of the seven enzymes were observed in moths. Soon after their appearance, bands were noted and a scan of the gel was done to conserve a permanent recording. For each population and each enzyme, a minimum of 30 adults was used.

Data analysis

Bands were interpreted in terms of loci and alleles and the allelic frequencies were determined. GENEPOP version 1.2 (Raymond & Rousset, 1995) was used to test for: (i) departures from the Hardy–Weinberg equilibrium (using Fisher's exact test); and (ii) the proportion of heterozygotes estimated under a Hardy–Weinberg equilibrium (He) and the proportion of heterozygotes observed (Ho). Differences among allelic frequencies for all pairs of populations were tested using Fisher's method, i.e. when genotypic frequencies were not independent, due to a linkage disequilibrium between loci, these were not maintained for the analysis of population structures. Deviations from equilibrium were estimated using Wright's F statistics (Weir & Cockerham, 1984). Values of Fis (deviation from random mating within a population), Fit (deviation from random mating in all populations) and Fst or 'fixation index' (deviation from random mating among populations) were given. The mean number of migrants (Nm) from each generation was estimated using the inverse relationship between Nm and Fst (Wright, 1951): $Nm = [(1/Fst) - 1]/4$, where N is the size of the population and m the mean migration ratio.

An unrooted neighbour-joining tree was constructed with the values of Fst among each population pair, using DARwin version 3.6.40 (Perrier & Jacquemoud-Collet, 2000).

Results

Of 21 enzymes screened in *P. xylostella*, four loci did not show a band: superoxide dismutase (EC 1.15.1.1), alcohol dehydrogenase (EC 1.1.1.1), glutamate dehydrogenase (EC 1.4.1.2) and phosphogluconate dehydrogenase (EC 1.1.1.44). Some loci had diffuse bands: xanthine dehydrogenase (EC 1.1.1.204), acid phosphatase (EC 3.1.3.2), adenylate kinase (EC 2.7.4.3), hydroxybutyrate dehydrogenase (EC 1.1.1.30), (S)-2-hydroxy-acid oxidase (EC 1.1.3.15), glucose dehydrogenase (EC 1.1.1.118), glutathione reductase (EC 1.6.4.2) and glucose-6-phosphate isomerase (EC 5.3.1.9). Sometimes, bands had a good resolution but loci appeared to be monomorphic in each population: glycerol-3-phosphate dehydrogenase (EC 1.2.1.12) and pyruvate kinase (EC 2.7.1.40). Enzymes with polymorphic loci that could be clearly interpreted were: isocitrate dehydrogenase (IDH, EC 1.1.1.42), $NADPH^+$ malate dehydrogenase (MDHP, EC 1.1.1.40), glucose-6-phosphate dehydrogenase (G6PDH, EC

Table 3. Allelic frequencies of the seven polymorphic loci in the 14 *Plutella xylostella* populations.

Locus		SA	BEN	BRA	FRA	JAP	USA	UZB	PHI	REU	AUAd	AUBr	AUMA	AUMe	AUSy
	N	32	31	30	30	33	35	32	34	32	33	34	35	35	35
	Allele														
IDHf	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.984	1.000	0.983	1.000	0.924	1.000	0.984	0.812	1.000	0.985	0.941	0.943	0.871	0.900
	3	0.016	0.000	0.017	0.000	0.076	0.000	0.016	0.188	0.000	0.015	0.059	0.057	0.129	0.100
IDHs	1	0.016	0.000	0.017	0.000	0.045	0.043	0.016	0.191	0.000	0.000	0.059	0.057	0.000	0.086
	2	0.984	1.000	0.983	1.000	0.955	0.928	0.937	0.794	1.000	0.970	0.941	0.929	1.000	0.914
	3	0.000	0.000	0.000	0.000	0.000	0.029	0.047	0.015	0.000	0.030	0.000	0.014	0.000	0.000
MDHPf	1	0.433	0.383	0.517	0.383	0.576	0.469	0.464	0.375	0.219	0.803	0.206	0.000	0.500	0.500
	2	0.567	0.617	0.483	0.617	0.424	0.531	0.536	0.625	0.781	0.197	0.794	1.000	0.500	0.500
MDHPs	1	0.203	0.339	0.167	0.100	0.182	0.057	0.000	0.221	0.047	0.470	0.044	0.057	0.029	0.071
	2	0.484	0.548	0.550	0.467	0.455	0.586	0.516	0.632	0.406	0.485	0.897	0.943	0.971	0.929
	3	0.313	0.113	0.283	0.433	0.363	0.357	0.484	0.147	0.547	0.045	0.059	0.000	0.000	0.000
G6PDH	1	0.078	0.210	0.217	0.100	0.000	0.029	0.125	0.103	0.094	0.030	0.191	0.043	0.029	0.143
	2	0.359	0.371	0.367	0.350	0.227	0.143	0.234	0.279	0.297	0.318	0.279	0.329	0.200	0.343
	3	0.422	0.419	0.416	0.550	0.697	0.529	0.547	0.618	0.563	0.652	0.471	0.500	0.557	0.514
	4	0.141	0.000	0.000	0.000	0.076	0.300	0.094	0.000	0.047	0.000	0.059	0.129	0.214	0.000
MPI	1	0.141	0.113	0.000	0.033	0.000	0.100	0.000	0.059	0.031	0.000	0.015	0.257	0.000	0.043
	2	0.141	0.113	0.200	0.300	0.455	0.129	0.188	0.221	0.141	0.318	0.088	0.357	0.014	0.114
	3	0.359	0.323	0.333	0.300	0.545	0.214	0.531	0.265	0.344	0.500	0.882	0.329	0.714	0.443
	4	0.203	0.290	0.317	0.200	0.000	0.457	0.188	0.412	0.250	0.152	0.015	0.043	0.229	0.300
	5	0.078	0.129	0.100	0.117	0.000	0.086	0.031	0.044	0.109	0.030	0.000	0.000	0.043	0.100
	6	0.078	0.032	0.050	0.050	0.000	0.014	0.063	0.000	0.125	0.000	0.000	0.014	0.000	0.000
PGM	1	0.016	0.016	0.017	0.000	0.000	0.014	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.062	0.032	0.117	0.017	0.000	0.114	0.047	0.000	0.016	0.031	0.000	0.000	0.014	0.043
	3	0.656	0.597	0.583	0.450	1.000	0.715	0.656	0.647	0.734	0.939	0.985	0.986	0.986	0.671
	4	0.250	0.307	0.200	0.533	0.000	0.143	0.187	0.235	0.219	0.030	0.015	0.014	0.000	0.200
	5	0.016	0.048	0.083	0.000	0.000	0.014	0.094	0.118	0.031	0.000	0.000	0.000	0.000	0.086
HK	1	0.000	0.097	0.000	0.050	0.000	0.071	0.031	0.015	0.031	0.000	0.103	0.000	0.014	0.014
	2	0.828	0.790	0.900	0.900	0.424	0.843	0.844	0.926	0.844	0.697	0.735	0.800	0.700	0.757
	3	0.172	0.113	0.100	0.050	0.576	0.086	0.125	0.059	0.125	0.303	0.162	0.200	0.286	0.229
AAT	1	0.234	0.452	0.417	0.100	0.000	0.057	0.016	0.471	0.422	0.091	0.000	0.186	0.086	0.214
	2	0.766	0.532	0.583	0.850	0.803	0.943	0.984	0.529	0.578	0.909	0.897	0.814	0.914	0.686
	3	0.000	0.016	0.000	0.050	0.197	0.000	0.000	0.000	0.000	0.000	0.103	0.000	0.000	0.100

See tables 1 and 2 for abbreviations.
N, number of adults analysed for each population.

1.1.1.49), mannose-6-phosphate isomerase (MPI, EC 5.3.1.8), phosphoglucomutase (PGM, EC 5.4.2.2), hexokinase (HK, EC 2.7.1.1) and aspartate aminotransferase (AAT, EC 2.6.1.1).

Some enzymes had several loci: IDH with loci IDHf and IDHs; MDHP with loci MDHPf and MDHPs. Allelic frequencies obtained for each locus in each population are given in table 3. Tests of the Hardy–Weinberg equilibrium showed that populations of *P. xylostella* were unbalanced at numerous loci. Frequencies observed differed from those estimated in 70 of the 126 comparisons ($P < 0.05$). They were caused by heterozygote deficits in the loci IDHs, MDHPs, G6PDH and MPI, and by an excess of heterozygotes in locus AAT (table 4). Fis values were significantly different from 0 for the loci MDHPs ($\chi^2 = 194.4$, df = 28, $P = 0.000$), G6PDH ($\chi^2 = 268.2$, df = 28, $P = 0.000$), MPI ($\chi^2 = \infty$, df = 28, $P = 0.000$), and AAT ($\chi^2 = 96.1$, df = 26, $P = 0.000$).

The loci IDHf and IDHs were monomorphic in populations of *P. xylostella* from Benin, France, Reunion Island and Melbourne. A low number of heterozygotes was observed for the locus PGM: it was monomorphic in the population from Japan and some of its alleles had low frequencies in populations from Adelaide, Brisbane, Mareeba and Melbourne in Australia. Allelic frequencies of the locus MDHPf showed variation between relatively close populations such as those from Adelaide and Mareeba. The mean heterozygosity observed (Ho) ranged from 0.183 (Brisbane) to 0.424 (Philippines). These values were lower than those estimated from the allelic frequencies, following the Hardy–Weinberg equilibrium (He), except for the populations from Melbourne, Sydney and Adelaide (table 5).

Allelic frequencies were significantly different for all pairs of populations using the Fisher test, except for the pair Benin/Brazil ($\chi^2 = 28.029$, $P = 0.06162$).

Some loci showed unbalanced linkages: IDHs and IDHf, MEf and G6PDH, MEf and MPI. Loci IDHf and MEf were not used in later analyses.

For all populations, $Fst = 0.103 \pm 0.025$, and $P(Fst = 0) < 0.001$. Values of Fst obtained for pairs of populations ranged from 0 (Benin–Brazil) to 0.230 (France–Melbourne) (table 6). In the Australian population group, Fst ranged from 0.039 (Brisbane–Melbourne) to 0.126 (Adelaide–Brisbane). The values of Fst between the populations from Australia and the other populations were over 0.100, except for the population from Sydney (table 6). Fst calculated for the population of Japan was also relatively high, it ranged from 0.072 (Japan–Adelaide) to 0.195 (Japan–Philippines). The estimated Fst for other pairs

Table 4. F statistics for all *Plutella xylostella* populations.

Locus	Fis	Fst	Fit
IDHf	−0.001	0.056	0.047
IDHs	0.103	0.049	0.147
MDHPf	−0.024	0.140	0.120
MDHPs	0.191*	0.173	0.331
G6PDH	0.484*	0.020	0.494
MPI	0.227*	0.092	0.298
PGM	0.008	0.136	0.144
HK	0.026	0.087	0.111
AAT	−0.417*	0.155	−0.197
All	0.151*	0.103*	0.238

See table 2 for abbreviations.

Table 5. Mean observed and expected heterozygosity at seven loci in *Plutella xylostella* populations.

Population	Ho	He	χ^2	df	*P*
South Africa	0.315	0.425	∞	14	0.000
Benin	0.406	0.436	73	14	0.000
Réunion Island	0.323	0.384	95.1	14	0.000
Brazil	0.374	0.429	77.2	14	0.000
USA	0.311	0.373	70	16	0.000
France	0.289	0.378	70.8	14	0.000
Uzbekistan	0.271	0.363	63.6	14	0.000
Japan	0.307	0.350	57.2	16	0.000
The Philippines	0.424	0.451	93.1	18	0.000
Australia					
Adelaide	0.316	0.310	45.7	16	0.000
Brisbane	0.183	0.253	50	16	0.000
Mareeba	0.222	0.261	34	14	0.002
Melbourne	0.314	0.274	62.6	14	0.000
Sydney	0.409	0.406	89.4	18	0.000

Ho, observed heterozygosity; He, estimated heterozygosity under Hardy–Weinberg equilibrium.

of populations ranged from 0.007 (Brazil–South Africa) to 0.104 (Uzbekistan–Philippines). The genetic differences between the populations were shown in an unrooted tree calculated with the values of Fst, using the neighbour-joining method (fig. 1). No relationship was observed between the geographic distances and the values of Fst. Populations from Benin and Brazil exhibited no differences (Fst = 0.0002), or were not considered as under reproductive isolation; whereas populations from Australia and Japan had a higher value of Fst, suggesting non-random mating and a low degree of gene flow with other populations. When populations from Australia and Japan were excluded from the analysis, Fst was 0.047 ± 0.020, with P (Fst = 0) < 0.001.

Populations differed the most about the loci MDHPs (Fst = 0.173), PGM (Fst = 0.136) and AAT (Fst = 0.155). Of the loci analysed, AAT was the only one that exhibited an excess of the heterozygotes observed, compared to the estimate. To assess its impact on the values of Fst, analyses were conducted without the allelic frequencies of AAT. The values of the heterozygosity observed were inferior to the values expected from the allelic frequencies, except in the population from Adelaide in Australia (table 7). The estimate of Fst for all the populations was 0.095 ± 0.027, with P (Fst = 0) < 0.033. Concerning population pairs, Fst ranged from 0 (Benin–Brazil) to 0.241 (France–Melbourne). The values of Fst were within the same estimates as previous analyses, but lower in the majority of the pairs of populations. When the populations from Australia and Japan were excluded from the analysis, the mean Fst = 0.02. However, the populations from Australia and Japan remained very different from the other populations.

The estimated comparative numbers of migrants per generation each year (Nm) has been calculated for some populations. When a population is in a suitable environment (25°C), a new generation is produced every four weeks. Nm was from 2 to 6 between populations in Australia and was estimated at 15 between populations from Benin and South Africa. The mean number of migrants was around seven individuals per generation for the populations from France and Uzbekistan. The mean numbers of migrants estimated between widely separated populations were not significantly different. The South African population exhibited a relatively high migration potential.

Discussion

The enzymes corresponded to 11 loci of which nine were polymorphic. *Plutella xylostella* can therefore be considered a highly polymorphic species. No alleles were found that discriminated one population from another. The analyses did not reveal a locus linked to the sex, or the presence of null alleles.

Table 6. Fst estimated for all pairs of *Plutella xylostella* populations.

Population	SA	BEN	BRA	FRA	JAP	USA	UZB	PHI	REU	AUAd	AUBr	AUMa	AUMe
BEN	0.017												
BRA	0.007	0.000											
FRA	0.024	0.064	0.043										
JAP	0.124	0.186	0.175	0.185									
USA	0.032	0.093	0.067	0.067	0.166								
UZB	0.026	0.102	0.063	0.036	0.122	0.035							
PHI	0.042	0.019	0.013	0.079	0.195	0.081	0.104						
REU	0.016	0.040	0.013	0.049	0.150	0.068	0.052	0.046					
AUAd	0.067	0.101	0.107	0.139	0.072	0.117	0.098	0.116	0.127				
AUBr	0.136	0.180	0.173	0.217	0.166	0.176	0.119	0.196	0.197	0.126			
AUMa	0.086	0.121	0.117	0.169	0.151	0.115	0.128	0.113	0.146	0.097	0.103		
AUMe	0.131	0.176	0.172	0.230	0.175	0.135	0.132	0.180	0.193	0.124	0.039	0.081	
AUSy	0.047	0.044	0.043	0.097	0.159	0.082	0.085	0.044	0.092	0.096	0.088	0.056	0.069

See table 1 for abbreviations.

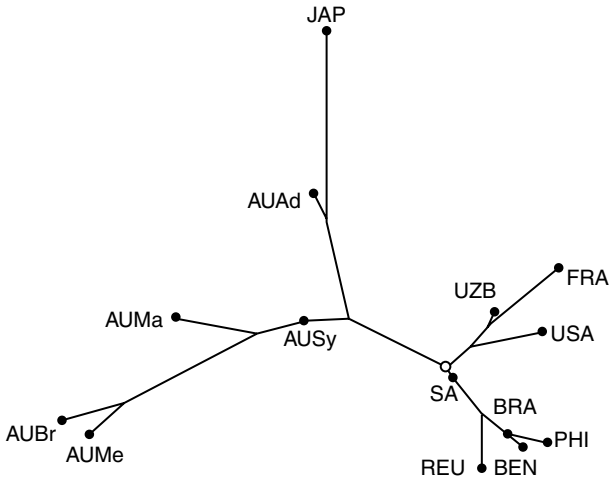

Fig. 1. Unrooted tree for fixation index (Fst) among pairs of populations of *Plutella xylostella*, calculated using the unweighted neighbour-joining method. See table 1 for abbreviations.

Table 7. Mean observed and expected heterozygosities at six loci (AAT excluded) in *Plutella xylostella* populations.

Population	Ho	He	χ^2	df	*P*
South Africa	0.365	0.489	∞	10	0.000
Benin	0.445	0.586	57.8	10	0.000
Réunion Island	0.400	0.523	53.2	10	0.000
Brazil	0.311	0.472	56	10	0.000
USA	0.457	0.523	59.5	10	0.000
France	0.320	0.531	63	10	0.000
Uzbekistan	0.370	0.438	37	10	0.000
Japan	0.394	0.449	43.9	10	0.000
The Philippines	0.350	0.501	56.6	12	0.000
Australia					
Adelaide	0.379	0.374	35	10	0.000
Brisbane	0.191	0.275	41.6	10	0.000
Mareeba	0.248	0.318	32.8	10	0.000
Melbourne	0.276	0.300	18.8	10	0.043
Sydney	0.324	0.418	40.1	12	0.000

Ho, observed heterozygosity; He, estimated heterozygosity under Hardy–Weinberg equilibrium.

Deviations from the Hardy–Weinberg equilibrium were caused by heterozygote deficits, except for one locus (aspartate aminotransferase) where an excess of heterozygotes was found. However, the results seemed to reveal a Walhund effect (resulting from a mix of several panmictic sub-populations for which initial allelic frequencies are fairly different) and may be explained by the various following causes which are not mutually exclusive. Departures from the Hardy–Weinberg equilibrium could be attributed to the sampling method, which included several populations of related species with different allelic frequencies. Another explanation was that the low heterozygosity could be derived from a small founding population. An example would be that a low number of migrants could have established a new population in an area. This hypothesis was an explanation for the reduction of the heterozygosity observed in the corn earworm *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Mallet *et al*., 1993). This phenomenon might have happened in the population from the USA since Geneva, New York is in northeastern USA, on the migration pathway of adults of *P. xylostella* from the southern states to Canada (Dosdall *et al*., 2002). Another example would be that climatic conditions could considerably reduce the size of a population in an area. In the northern region of Japan during winter, densities of *P. xylostella* are around five individuals to ten cabbage plants (Honda *et al*., 1992). In Benin, where cabbage production is maintained throughout the year, the population size decreases from June to September, because of the impact of heavy rains (Bordat & Goudegnon, 1997). A third explanation is that heterozygote deficits could be a consequence of reproductive isolation. Kim *et al*. (1999) considered this hypothesis, as they observed a high Fis in populations from South Korea. In the present study, there were not sufficient data to evaluate population dynamics throughout the year, or to ascertain the level of migration. Therefore, none of the three hypotheses could be confirmed.

The global Fst was relatively high (Fst = 0.103), compared to the values obtained in previous studies on *P. xylostella*. Caprio & Tabashnik (1992) obtained Fst values of 0.028–0.034 and Kim *et al*. (1999) estimated Fst = 0.0215. The differentiation index for populations excluding those from Australia and Japan (Fst = 0.047) was similar to these values. Values of Fst for populations of other lepidopterous species vary from 0.109 for pine beauty moth *Panolis flammea* (Denis & Schiffermüller) (Lepidoptera: Noctuidae) (Wainhouse & Juke, 1997), Fst = 0.080 for bog fritillary *Proclossiana eunomia* (Esper) (Lepidoptera: Nymphalidae) (Nève *et al*., 2000), Fst = 0.007 in the populations of the migrating black antworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) (Buès *et al*., 1994) and among African and European populations of the cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Nibouche *et al*., 1998).

In the present study, estimates of Fst between the populations were not correlated with the geographic distances between them. Populations from Australia and Japan were the most different, whereas populations from Brazil and Benin exhibited very similar allelic frequencies. This result has to be confirmed using DNA data to elucidate the phylogeny of these populations. Results of the enzyme electrophoresis were not informative enough to assume that these populations had the same geographical origin, or if the allelic frequencies were related to a high gene flow.

Gene flow between populations was limited, as confirmed by the number of migrants, particularly between populations in Australia. Nevertheless, migrations in tropical and subtropical areas (e.g. South Africa/Benin) appeared to be more important than in temperate areas (e.g. Uzbekistan/France).

Populations from Australia and Japan were different from other populations and between themselves. The mean number of migrants estimated among the Australian populations was reduced despite the short distances separating these populations. The differences could be the consequence of a small size of the population and a reduced gene flow. When populations are small, effects of genetic drift are more important. As a consequence, a higher number of migrants are necessary so gene flow can counterbalance genetic drift (Allendorf & Phelps, 1981). These populations could be in a condition of high reproductive isolation.

In addition, variations of the genetic structure of populations observed through their allelic frequencies could

be linked to insecticide resistance. The role of esterases in resistance to organophosphorous compounds has been demonstrated in *P. xylostella* (Liu *et al*., 1982; Miyata *et al*., 1986). In a study on the cotton whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), the allelic frequencies of esterases were different in an insecticide treated sample and in an unsprayed control sample (Wool *et al*., 1993). The enzymes studied here were not directly involved in the insecticide resistance mechanism. However, Herrero *et al*. (2001) has found a correlation between the presence of an allele in the locus MPI and the resistance to the toxin Cry1A of *Bacillus thuringiensis* (Bt) in *P. xylostella*. The change in frequency of the isozyme is an argument in favour of a linkage at the locus MPI to the Cry1A resistance locus. The appearance of this isozyme was caused by genetic selection and not by a physiological induction. As in the present study, no data on resistance in the populations sampled (to Bt toxins or other insecticides) were available, the link between the resistance and the allelic frequencies observed has not been confirmed. But, considering the results, it is possible that the isozyme linked to the locus of Bt resistance corresponded to the allele 1 or 2 of the locus MPI. The presence of this isozyme could be used to detect and to identify a resistance to Bt toxins in a strain and to evaluate its dispersal ability in the area. Because of the MPI linkage to *Bt* toxins, this locus might not be neutral, as required for a study of geographic variations. Results obtained in the present study show that populations are not most differentiated at this locus.

In conclusion, estimates of Fst suggested that the degree of differentiation could vary within a population of *P. xylostella* (migrations, reduction of size due to environmental conditions), and that gene flow could be important in tropical and sub-tropical populations. Diamondback moth population sizes can vary as a function of their local environment and migration in temperate areas where they probably do not overwinter. Gene flow could maintain the low level of allelic variation between most populations. Enzyme genotypes were not informative enough to precisely evaluate either the gene flow between populations a short distance apart or the gene flow over long distances, probably by way of mass migrations. Allelic frequencies may vary in time, according to population size, which itself varies due to genetic drift and migration. These factors could then change the frequency of enzyme genotypes.

Recent work on the genetic basis of insecticide resistance has shown that the frequencies of some isozymes could be related to a resistant genotype (Herrero *et al*., 2001). Further studies are needed to confirm this hypothesis and to consider enzyme genotypes as markers to detect resistance in a newly established population. The enzyme electrophoresis technique was not accurate enough to gain insights into the phylogeny of *P. xylostella*. Molecular markers are more powerful tools to assess relationships at the population level.

Acknowledgements

The authors thank A. Bordat for her collaboration in the experiments, and all the colleagues from overseas who collected samples. This study was supported in part by grants from the Centre de Coopération Internationale en Recherche Agronomique pour le Développement, France.

References

Allendorf, F.W. & Phelps, S.R. (1981) Use of allelic frequencies to describe population structure. *Canadian Journal of Fisheries and Aquatic Science* **38**, 1507–1514.

Bordat, D. & Goudegnon, A.E. (1997) The status of diamondback moth and its natural enemies in the Porto Novo and Cotonou areas in Benin. p. 312 *in* Sivapragasam, A., Loke, W.H., Hussan, A.K. & Lim, G.S. (*Eds*) *Proceedings of the Third International Workshop on the Management of Diamondback moth and other Crucifer Pests*, *29 October–1 November 1996*, Kuala Lumpur, Malaysia, Malaysian Agricultural Research and Development Institute.

Buès, R., Freuler, J., Toubon, J.F., Gerber, S. & Poitout, S. (1994) Stabilité du polymorphisme enzymatique dans les populations d'un Lépidoptère migrant, *Agrotis ipsilon*. *Entomologia Experimentalis et Applicata* **73**, 187–191.

Caprio, M.A. & Tabashnik, B.E. (1992) Allozymes used to estimate gene flow among populations of diamondback moth (Lepidoptera: Plutellidae) in Hawaii. *Annals of the Entomological Society of America* **21**, 808–816.

Chapman, J.W., Reynolds, D.R., Smith, A.D., Riley, J.R., Pedgley, D.E. & Woiwod, I.P. (2002) High-altitude migration of the diamondback moth *Plutella xylostella* to the U.K.: a study using radar, aerial netting, and ground trapping. *Ecological Entomology* **27**, 641–650.

Cheng, E.Y. (1981) Insecticide resistance study in *Plutella xylostella* L. I. developing a sampling method for surveying. *Journal of Agricultural Research of China* **30**, 277–284.

Daly, J.C. & Gregg, P. (1985) Genetic variation in *Heliothis* in Australia: species identification and gene flow in the two pest species *H. armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* **75**, 169–184.

Dosdall, L., Mason, P., Olfert, O., Kaminski, L. & Keddie, B. (2002) The origins of infestation of diamondback moth, *Plutella xylostella* (L.), in canola in western Canada., pp. 95–100 in *The management of diamondback moth and other crucifer pests: Proceedings of the fourth international workshop*, *26–29 November 2001*, Melbourne, Australia, Department of Natural Resources and Environment.

Harcourt, D.G. (1986) Population dynamics of the diamondback moth in southern Ontario. p. 3. *in* Talekar, N.S. & Griggs, T.D. (*Eds*) *Proceedings of the First International Workshop on the Diamondback Moth Management*, *11–15 March 1985*, Tainan, Taiwan, Asian Vegetable Research and Development Center.

Herrero, S., Ferre, J. & Escriche, B. (2001) Mannose phosphate isomerase isoenzymes in *Plutella xylostella* support common genetic bases of resistance to *Bacillus thuringiensis* toxins in lepidopteran species. *Applied and Environmental Microbiology* **67**, 979–981.

Hillis, D.M., Moritz, C. & Mable, B.K. (1996) *Molecular systematics*. 620 pp. Sunderland, Massachusetts, Sinauer.

Honda, K., Miyahara, Y. & Kegasawa, K. (1992) Seasonal abundance and the possibility of spring immigration of the diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Yponomeutidae) in Morioka City, Northern Japan. *Journal of Applied Entomology and Zoology* **27**, 517–525.

Kfir, R. (1998) Origin of the diamondback moth (Lepidoptera: Plutellidae). *Annals of the Entomological Society of America* **91**, 164–167.

Kim, Y., Kim, K. & Kim, N. (1999) Genetic difference between two field populations of *Plutella xylostella* (Linné) based

on four polymorphic allozymes. *Journal of Asia-Pacific Entomology* **2**, 1–5.

Lim, G.S. (1986) Biological control of diamondback moth. p. 159 *in* Talekar, N.S. & Griggs, T.D. (*Eds*) *Proceedings of the First International Workshop on the Diamondback Moth Management, 11–15 March 1985*, Tainan, Taiwan, Asian Vegetable Research and Development Center.

Liu, M.Y., Tzeng, Y.J. & Sun, C.N. (1982) Insecticide resistance in the diamondback moth. *Journal of Economic Entomology* **75**, 153–155.

Mackenzie, J.M.B. (1958) Invasion of diamondback moth (*Plutella maculipennis* Curtis). *Journal of Entomological Science* **91**, 247–250.

Mallet, J., Korman, A., Heckel, D.G. & King, P. (1993) Biochemical genetics of *Heliothis* and *Helicoverpa* (Lepidoptera: Noctuidae) and evidence for a founder event in *Helicoverpa zea*. *Annals of the Entomological Society of America* **86**, 189–197.

Miyata, T., Saito, T. & Noppun, V. (1986) Studies on the mechanism of diamondback moth resistance to insecticides. p. 347 *in* Talekar, N.S. & Griggs, T.D. (*Eds*) *Proceedings of the First International Workshop on the Diamondback Moth Management, 11–15 March 1985*, Tainan, Taiwan, Asian Vegetable Research and Development Center.

Nève, G., Barascud, B., Descimon, H. & Baguette, M. (2000) Genetic structure of *Proclossiana eunomia* populations at the regional scale (Lepidoptera, Nymphalidae). *Heredity* **84**, 657–666.

Nibouche, S., Buès, R., Toubon, J.F. & Poitout, S. (1998) Allozyme polymorphism in the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae): comparison of African and European populations. *Heredity* **80**, 438–445.

Pasteur, N., Pasteur, G., Bonhomme, F., Catalan, J. & Britton-Davidian, J. (1987) *Manuel technique de genetique par electrophorese des proteines*. 217 pp. Paris, Lavoisier.

Perrier, X. & Jacquemoud-Collet, J.P. (2000) DARwin 3.6.40 CIRAD-Flhor, Montpellier.

Raymond, M. & Rousset, F. (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248–249.

Sun, C.N., Wu, T.K., Chen, J.S. & Lee, W.T. (1986) Insecticide resistance in diamondback moth. p. 359 *in* Talekar, N.S. & Griggs, T.D. (*Eds*) *Proceedings of the First International Workshop on the Diamondback Moth Management, 11–15 March 1985*, Tainan, Taiwan, Asian Vegetable Research and Development Center.

Tabashnik, B.E., Cushing, N.L. & Johnson, M.W. (1987) Diamondback moth (Lepidoptera: Plutellidae) resistance to insecticides in Hawaii: intra-island variation and cross-resistance. *Journal of Economic Entomology* **80**, 1091–1099.

Talekar, N.S. & Shelton, A.M. (1993) Biology, ecology and management of the diamondback moth. *Annual Review of Entomology* **38**, 275–301.

Tsunoda, S. (1980) Eco-physiology of wild and cultivated forms in *Brassicas* and allied genera. pp. 109–120 *in* Tsunoda S., Hinata, K. & Gomez-Campo, C. (*Eds*) *Brassica crops and wild allies: biology and breeding*. Japan Scientific Societies Press.

Wainhouse, D. & Juke, M.R. (1997) Geographic variation within populations of *Panolis flammea* (Lepidoptera: Noctuidae) in Britain. *Bulletin of Entomological Research* **87**, 95–99.

Weir, B.S. & Cockerham, C.C. (1984) Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.

Wool, D., Gerling, D., Bellotti, A.C. & Morales, F.J. (1993) Esterase electrophoretic variation in *Bemisia tabaci* (Genn.) (Hom., Aleyrodidae) among host plants and localities in Israel. *Journal of Applied Entomology* **115**, 185–196.

Wright, S. (1951) The genetical structure of populations. *Annals of Eugenics* **15**, 323–354.

(Accepted 13 October 2005)